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STUDY OF SILK GLANDS AND SILK SECRETING APPARATUS IN *Nephila pilipes*

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ABSTRACT

The most important characteristic feature of spider is their ability to produce silken threads of various types. Such specialization of fiber types has occurred through the evolution of different silk producing glands which functions as small biofactories.

In giant wood spider *Nephila pilipes*, we found five types of silk glands located inside the abdomen as – two pairs of ampullate glands, three pairs of cylindrical glands, three pairs of aggregate glands, numerous aciniform glands in four clusters and many pyriform glands in two clusters. Each gland leads to specific silk secreting outlet (spinnerets) which opens to outside in the form of tiny spigots. In *Nephila pilipes*, three pairs of spinnerets are present ventrally at the end of abdomen.

In the present investigation, attempt have been made to explain the external morphology of various silk glands and details of spinning field with the help of scanning electron micrograph (SEM) of spinnerets of *Nephila pilipes*.
Key words: *Nephila pilipes,* silk glands, spinnerets, SEM.

INTRODUTION

The production of silk is widespread among animal kingdom but it is particularly associated with phylum arthopoda (Lucas and Ruddal, 1968). Although certain insects (eg. some lepidoptera, hymenoptera and neuroptera) are capable of silk production, this ability is usually restricted to a single stage in their life span such as build cocoon prior to pupation. In contrast, all spiders possess spinning glands which they use not only for making egg sac and draglines but also for building snares (Foelix, 1996).

The silk glands of spiders are located in the abdomen. These glands are mainly tubular, ending blindly inside and outside by means of spigots present on the spinnerets.

MATERIALS AND METHODS

The adult females of *Nephila pilipes* were collected from the woods of Melghat (India) in the month of October and November. These spiders are most easily recognized by their special web building habit. Their orb webs are placed in

opening between trees and shrubs where insects are likely to fly. Spiders are kept in suitable plastic container to which holes were made for ventilation.

Spider was dissected under Magnus Steriozoom binacular Microscope with camera attachment. Photographs of ampullate and cylindrical glands were taken with FUJIFILM FINEPIX 52000 HD 10.0 megapixel 15X wide digital camera. Photos of aggregate pyriform and aciniform glands were taken with camera attached to micro scope.

Spinnerets of freshly killed spider were prepared for examination by SEM. Procedure was performed as described by Townley and Tillinghast (2009) with a few exceptions. Dehydration through the ethanol series (30 %, 50 %, 70 %, 90 % and twice in 100 %) proceeded at a slower rate. Abdomen squeezed by forceps was allowed to air dry.

Spinnerets were mounted on stub which is covered with double sided carbon tapes. Gold coating was done with Auto Fine Sputter Coater (Model No. JEDL JFC –1600) at current 20 mA and pressure 20 Pa.

SEM photographs were taken on Digital Scanning Electron Microscope (Model No. JEOL JSM -6380A) at Visveswaraiya National Institute of Technology, Nagpur, India. Images are viewed with a voltage of 5 kV under low vacuum. Multiple images of each spinneret were taken at various magnifications and from different prospective to reduce the probability of missing any spigot or spinning tubes. The identification of various spigots on spinnerets was done using rules given by Coddington (1989).

The following anatomical abbreviations are used in the text and figures as given by Kutner (2007). AC – aciniform gland spigot(s); AG – aggregate gland spigot(s); ALS – anterior lateral spinnerets; AMP – ampulla; AT – anal tubercle; C – comb hairs; Co – colulus; Cy - cylindrical gland spigot(s); DT – duct; MAP - major ampullate gland spigot(s); mAP – minor ampullate gland spigot(s); Pi – pyriform gland spigot(s); PLS – posterior lateral spinnerets; PMS – posterior median spinnerets; SR - secretory region.

RESULTS AND DISCUSSION

Nephila pilipes possesses highly elaborated arrangement of spinning glands. The morphological feature such as the shape of gland is one of the important criterions in classification of glands.

Ampullate glands: These are the most prominent silk glands and constitute the major part of the abdominal viscera. These are found in two pairs, one pair of major ampullate gland and a pair of minor ampullate gland. Each ampullate gland has three regions viz. an anterior tubular highly coiled part with a main function of secreting silk is called secretory region (SR), the anterior duct enlarges posteriorly into a wide sac forming ampulla and hence getting the name ampullate (AMP). The ampulla is the largest portion storing mainly silk in liquid form. The ampulla leads

into a long tubular posterior duct (DT) which takes several 'U' turns throughout its length, leading to spigot. The increase in the length of DT is probably due to the absorption of water from the liquid silk and to undergo transformation, polymerization and orientation of the silk as suggested by Kovoor and Zylberberg (1972).

The entire major ampullate gland measures 30-35 mm when fully stretched. The ampulla has diameter of 1.5-2 mm at its widest part. However volume of ampulla depends upon the storage of secretory products (Plate 1.A and B). Further, the presence of silk fully filled in the ampulla at various stages of development signifies that the silk is continuously produced and utilized. The minor ampullate gland resembles more or less that of major ampullate glands. The colour of ampulla as in previous one is yellow. In the mature female, minor ampullate gland measures 25-30 mm and ampulla has maximum diameter 1-1.5 mm (Plate 1 A and C).

Yellow colour of the secretion of these glands and the yellowish tinge of the dragline, framework and the radii of the web prove that these parts are made of the silk from ampullate glands.

Ampullate glands are very well developed in adult spider but the reservoirs are very much reduced in gravid females. Ampullate glands slowly undergo reduction in size as ovary reaches maturity. During the fortnight before laying eggs, the spider neither construct a new web nor tries to repair damaged part of the old web. This behavior is related with the reduction in size of ampullate glands in gravid females. Thus, these glands play a major role in construction of web.

Aciniform glands: Aciniform glands are minute, numerous and are arranged in four clusters. These are situated in front of middle and hind spinneret, a separate cluster for each of the spinnerets (Plate 1-D). The cluster of the middle spinneret consists of few glands each while those of hind spinneret contain a large number of them. These glands are whitish, having a swollen base in the form of bulb or acinus and hence the name. Each gland is supplied with a separate duct that opens to exterior through the spigots on PMS and PLS.

The secretion of these silk glands is utilized in covering the insects which is well known as 'swathing band'(Ramkrishna and Tikader, 1988).

Aggregate glands: During present investigation three pairs of aggregate glands are found. These glands are located superficially in the body cavity in close association with hepato pancreatic mass and the ampullate glands. Glands are named so because of their tree like branched form. Each gland consists of numerous lobed branches (Plate 1-E). The lobes are irregular shape and the glands nearly run the entire length of the abdomen. Posteriorly, the glands lead to a much convoluted duct that opens through a spigot of pointed apex.

As the spiders mainly rely on insects for food, various modes of capturing insects are adopted by spiders. These are achieved by producing some sticky spiral threads, which are very well developed in orb weaver like *Nephila pilipes*. Aggregate

glands are typical of araneids. They produce glue substance for the web catching spiral (Foelix, 1996).

Pyriform glands: there are numerous minute pyriform glands arranged in two clusters, situated in the posterior region of abdomen, each in front of the respective ALS. These are more or less pear shaped and hence get the name pyriform gland (Plate:2-F). Each gland is provided with a separate duct that opens to the exterior through spigot on ALS. Silk of these glands is used for attachment discs at junctions.

Cylindrical glands: Three pairs of these glands are found along the ventrolateral sides of the body cavity and runs antero-posteriorly (Fig.2-G). The name cylindrical gland was employed because of cylindrical form of gland (Fig.1-H). The gland is uniform in diameter about 0.5-1mm. The anterior end bends in posterior direction to form a loop. Posteriorly, the gland continues into a duct that opens to exterior through a spigot with broad mouth.

The gland appears red in colour due to red colour of the secretion. The morphology of gland is more clear before the deposition of eggs by the female and diminishes in size after formation of egg sac (Plate:2 -I). The pink colour of the silk used for egg sac confirms that the silk of these glands is employed for the construction of egg sac. These glands are not seen in males and immature females. These glands are found only in adult female spider, their size and secretory activity increase with the development of eggs in the ovaries (Gray, 1991).

SILK SECRETING APPARATUS

The number and arrangement of the abdominal appendage viz. spinnerets forms the part of taxonomic importance (Ramkrishna and Tikader,1988). *Nephila pilipes* is an opisthothelae spider. The terminal part of each spinneret is provided with spigots forming the spinning field. The spigot morphology corresponds to the web type of spider (Mathew *et al.,* 2011). In *Nephila pilipes*, the six spinnerets, colulus and anal tubercle are well formed distinct structure (Plate:3-A).

ALS is broad, short and two segmented. The basal segment is hard chitinous ring clothed with hairs. The base of terminal segment chitinised into a ring to which is attached a muscle that brings about the movement of segment (Thakur and Tembe, 1956). Pyriform field is uniform across the ALS tip. There are numerous spigots through which open the pyriform glands (Plate:2- B, D). A major ampullate spigots is found on mesal margin of ALS (Plate:3- B, C)

The PMS is narrow, conical and consists of a single segment. It bears three classes of spigots: single posterior minor ampullate spigot, single cylindrical gland spigot and three to four small aciniform gland spigots (Plate:3-E, F). Posterior to MAP spigot, there appears vestigial spigots on the PMS. It is probably the vestigial remnants of minor ampullate gland spigot which is lost in the adult instar (Plate:3-G).

The PLS is quite complex. On its mesal basal margin two cylindrical gland spigots are present. The spigots of aggregate glands are observed on anterolateral margin. Across the PLS tip, a second group of aciniform gland spigots are seen (Plate:3-H, I).

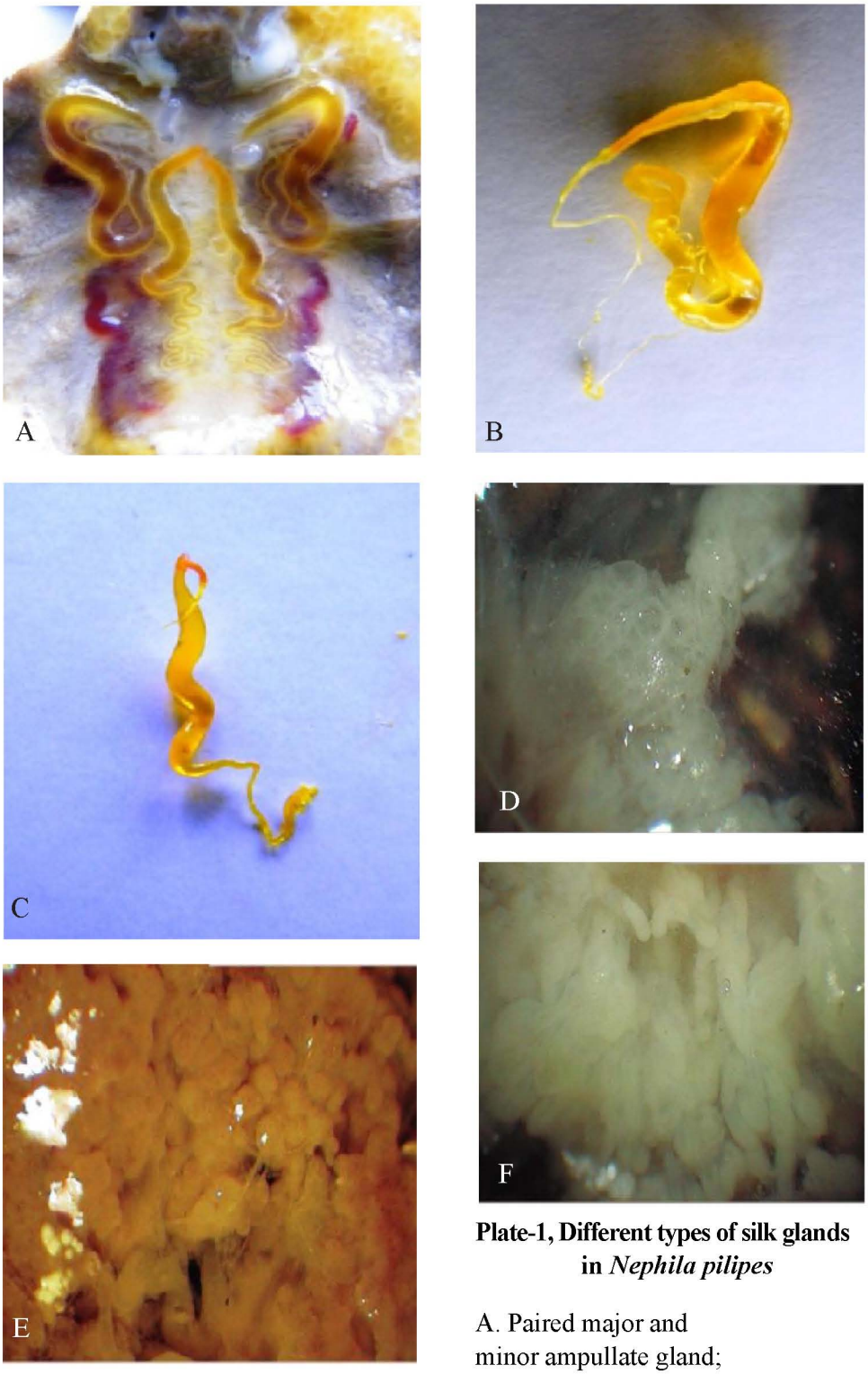

Plate-1, Different types of silk glands in *Nephila pilipes*

A. Paired major and minor ampullate gland; B. Major ampullate gland; C. Minor ampullate gland D. Aciniform gland E. Aggregate glands; F. Pyriform glands;

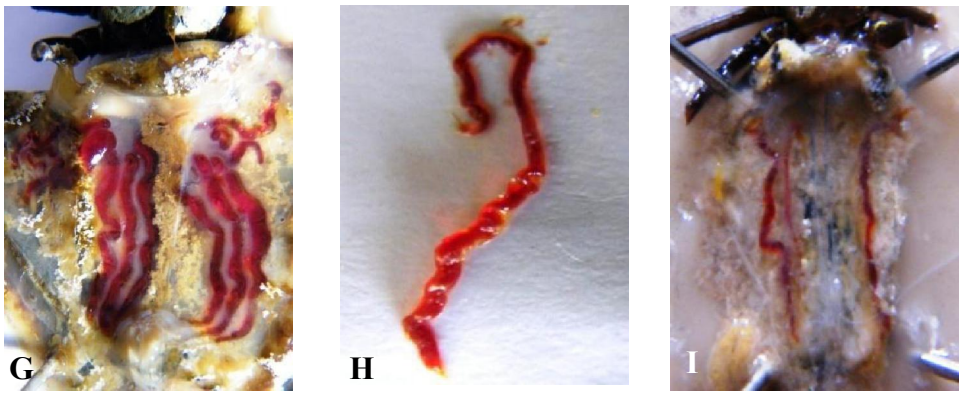

Plate:2, G. Three pairs of cylindrical glands in gravid female; H. Single cylindrical gland; I. Cylindrical glands in gravid female after egg laying.

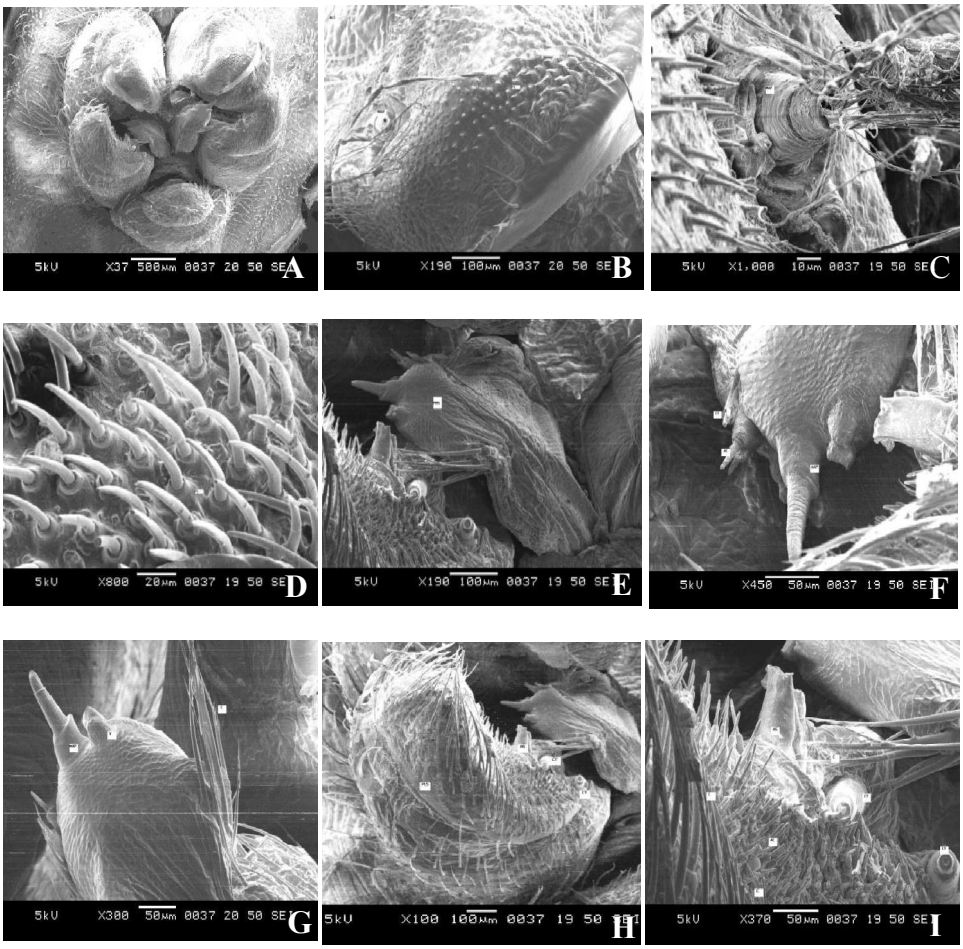

Plate:3, Spinning field of adult female *Nephila pilipes*
A.Spinnerets, colulus and anal tubercle; B. ALS showing MAP and Pi; C. ALS showing MAP; D. ALS showing Pi; E.PMS; F. PMS showing MAP, CY, AC; G. PMS showing C and N; H. PLS showing CY, AG, AC; I. PLS showing CY, AG, AC and C.

SEM micrographs of PMS and PLS (Plate:3-G, I) shows comb like spines. These may help the spider to position and press and together silk spun from spigot while draw down in air (Knight and Vollrath, 2002).

CONCLUSION

The giant wood spider, *Nephila pilipes***,** builds their orb web having geometrical precision and beauty. This masterpiece of their craftsmanship is a result of the secretion of various silk glands that comes out from minute microscopic spigots. Spinning behavior obviously depend to some extent on spinnerets and spigots in spinning field.

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